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# Dysregulation of LDL receptor under the influence of inflammatory cytokines: A new pathway for foam cell formation[1]

**XIONG Z. RUAN, ZAC VARGHESE, STEPHEN H. POWIS, and JOHN F. MOORHEAD**

*Centre for Nephrology, Royal Free and University College Medical School, University College London, London, England, United Kingdom*

**Dysregulation of LDL receptor under the influence of inflammatory cytokines: A new pathway for foam cell formation.**

*Background.* Lipid-mediated renal injury is an important component of glomerulosclerosis and its similarity to atherosclerosis is well described. This study focused on the relationship between lipid-mediated injury and inflammation by examining the role of inflammatory cytokines in the regulation of human mesangial cell low-density lipoprotein (LDL) receptors.

*Methods.* A human mesangial cell line (HMCL) was used to study the effects of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) on the regulation of LDL receptor mRNA and protein in the presence of a high concentration of native LDL (250 μg/mL).

*Results.* Native LDL caused foam cell formation in HMCL in the presence of antioxidants, TNF-α and IL-1β. Both cytokines overrode LDL receptor suppression induced by a high concentration of LDL and increased LDL uptake by enhancing receptor expression. These cytokines also caused increased expression of SCAP [sterol responsive element binding protein (SREBP) cleavage activation protein], and an increase in the nuclear translocation of SREBP, which induces LDL receptor expression.

*Conclusion.* These observations demonstrate that inflammatory cytokines can modify cholesterol-mediated LDL receptor regulation in mesangial cells, permitting unregulated intracellular accumulation of unmodified LDL and causing foam cell formation. These findings suggest that inflammatory cytokines contribute to lipid-mediated renal damage, and also may have wider implications for the study of inflammation in the atherosclerotic process.

The low-density lipoprotein receptor (LDLr) is the primary receptor for binding and internalization of plasma-derived LDL-cholesterol and regulates plasma LDL concentrations [1, 2]. Brown and Goldstein observed that LDL receptor activity is under tight metabolic control from intracellular cholesterol concentration through a feedback system [2]. This system maintains a constant level of cholesterol in hepatocytes and other cells by controlling both the rate of cholesterol uptake from LDL and the rate of cholesterol synthesis [1]. Therefore, native LDL is ineffective in generating lipid rich foam cells that are precursors of fatty streaks. In contrast, modified LDL (oxidized or glycosylated) contributes to atherogenesis through its uncontrolled uptake via scavenger receptors.

Intracellular cholesterol regulates LDL receptor gene transcription through the LDL receptor promoter, which contains three imperfect direct repeats of 16 bp and two TATA-like sequences of 7 bp each. Repeats one and three have some homology with the GC-rich region that binds a transcription-activator protein termed SP-1, and repeat 2 is a sterol regulatory element (SRE-1) binding point. The sterol regulatory element binding proteins, SREBP-1 and SREBP-2, are central molecules in the cholesterol feedback system. In sterol-depleted cells, a two-step cleavage process releases the NH2-terminal segment of SREBP, which then enters the nucleus, binds to LDL receptor promoter region, and activates gene transcription [3]. The process begins when a protease cuts SREBP-2 at site 1, which is at or near an arginine in the luminal loop, thereby separating the two membrane-spanning segments. This allows a second protease to cut the protein at site 2, which is in the middle of the first transmembrane segment, releasing the N-terminal domain into the cytosol. The site 1 protease, which is strictly regulated by sterols, is active in sterol-depleted cells and turned off when sterol accumulates. The site 2 protease is not regulated directly by sterols, but can act only after the site 1 protease. SREBP cleavage-activating protein (SCAP) has recently been identified in the hamster and in humans

[1] **See Editorial by van Zonneveld and Rabelink, p. 2037**

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as a key molecule [4] in the regulation of cholesterol metabolism by stimulating cleavage of SREBP-1 and -2. It has multiple membrane-spanning regions, five of which resemble the sterol-sensing domain of 3-hydroxy-3-methylglutaryl coenzyme A (HMGCo-A) reductase, an endoplasmic reticulum enzyme whose degradation is accelerated by sterols. A mutation at codon 443 of SCAP enhances the cleavage-stimulating ability of SCAP and renders LDL receptor in Chinese hamster ovary (CHO) cells resistant to inhibition by sterols suggesting that SCAP may be a key factor in the feedback regulation of LDL receptor [4]. Therefore, SCAP appears to act as a "cholesterol sensor" in mammalian cells [4, 5].

We have previously established that mesangial cells are closely related to vascular smooth muscle cells and have both LDL receptor and scavenger receptors [6, 7]. Many of the histological features of progressive glomerular and tubulointerstitial renal disease and chronic renal transplant dysfunction share molecular mechanisms that are considered important in the pathogenesis of atherosclerosis [8]. The term "glomerular atherosclerosis" has been proposed on the basis of these observations. Recent experimental evidence suggests that inflammation is an aggravating factor in atherogenesis [9]. Elevated plasma levels of cytokines including tumor necrosis factor-α (TNF-α), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF) and interleukin-1 (IL-1) also have been found in various kidney diseases, suggesting that inflammatory cytokines may contribute to glomerulosclerosis [10, 11]. However, the mechanism by which inflammation leads to glomerular atherosclerosis is unclear. Our recent work has shown that TNF-α, TGF-β, PDGF, and IL-1β increase LDL receptor gene expression through a sterol- and proliferation-independent gene transcription pathway in a human mesangial cell (HMC) line. This suggests that inflammatory cytokines may increase accumulation of cholesterol through the dysregulation of LDL receptor in human mesangial cells, which may be important in the progression of renal dysfunction [12]. In this study, we investigated the molecular mechanisms by which inflammatory cytokines increase LDL receptor expression in the presence of a maximally suppressive concentration of unmodified native LDL.

## METHODS

### Cell culture

An established stable human mesangial cell line cells (HMCL) was used in all experiments (kindly donated by Dr. J.D. Sraer, Hôpital Tenon, Paris, France). HMCs were immortalized by transfection with T-SV40 and H-ras oncogene, retaining many of the morphological and physiological features of normal HMCs [12, 13]. These cells were cultured in growth medium containing Roswell Park Memorial Institute RPMI-1640 medium, 5% fetal calf serum (FCS), 2 mmol/L glutamine, 100 unit/mL penicillin, 100 μg/mL streptomycin, 2.5 μg/mL amphotericin, 5 μg/mL insulin, 5 μg/mL human transferrin, and 5 ng/mL sodium selenite. For the primary culture of HMCs, above culture medium was used with 20% FCS. All experiments were carried out in serum-free RPMI-1640 medium containing 0.2% bovine serum albumin (BSA) with the anti-oxidants ethylenediaminetetraacetic acid (EDTA) and butylated hydroxytoluene (BHT) at final concentrations of 100 and 20 μmol/L, respectively (Sigma, Poole, Dorset, UK). All reagents for cell culture were obtained from Gibco BRL (Paisley, UK). Recombinant TNF-α (2.0 to $5.0 \times 10^7$ U/mg) and IL-1β (1.0 to $3.3 \times 10^8$ U/mg) were obtained from R&D Systems (Europe Ltd., Abingdon, UK).

### Preparation of lipoprotein

Plasma was collected from healthy human volunteers and LDL was isolated by sequential ultracentrifugation [12]. The extent of lipid peroxidation of the LDL was estimated as the concentration of thiobarbituric acid reactive substances (TBARS) as described previously [6] and the results expressed as nmol of malondialdehyde per mg LDL (nmol MDA/mg LDL). The levels of TBARS in native LDL used in the study were less than 0.1 nmol MDA/mg LDL. LDL labeled with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI-LDL) was obtained from Biogenesis (Poole, UK).

### Morphological examination

Human mesangial cell lines were plated in chamber slides for tissue culture (Nunc Inc., Naperville, IL, USA) and incubated in serum-free RPMI-1640 medium with native LDL or cytokines plus native LDL in the absence or presence of 250 μg/mL of polyinosinic acid (PolyI) or 5 mg/mL of heparin. After 24 hours of incubation, the cells were washed three times with phosphate-buffered saline (PBS), fixed for 30 minutes with 5% formalin solution in PBS, stained with Oil Red O for 30 minutes and counterstained with hematoxylin for another five minutes. Finally, the cells were examined by light microscopy.

### Cell labeling and flow cytometric analysis

Human mesangial cell lines were incubated in serum-free medium alone or with 50 ng/mL of TNF-α or 5 ng/mL of IL-1β in the absence or presence of a high concentration of native LDL (250 μg/mL) for 24 hours, respectively, then the medium was replaced by fresh serum-free medium containing 10 μg/mL DiI-labeled LDL for five hours at 37°C. The cells were detached from the plates by incubation with 0.5% trypsin-EDTA and fixed in 5% formalin solution in PBS. The fixed cells were washed three times in PBS and analyzed by fluorescence-activated cell sorter analysis (FACS) using a flow cytometer (Coulter, EPICS XL-MCL). Forward angle and 90° light

scatter gates were established to exclude dead cells and cell debris from the analysis. Fluorescence signals from the accumulated DiI in the cells were collected at 555 to 600 nm by a photomultiplier, converted to digital format and processed for storage and display in one-parameter log scale frequency histograms. Five thousand cells were analyzed in each sample. The data were evaluated by mean fluorescence intensity (MFI). Autofluorescence signals from unlabeled cells were used as negative controls in each experiment. The MFI of the DiI-labeled cells was calculated by subtracting the autofluorescence intensity from the observed MFI of labeled cells. The average of the duplicate determination was used for statistical analyses, and each experiment was carried out in duplicate.

### Northern blot analysis

Total RNA was isolated from cultured HMCL by the guanidinium method. For Northern blot analysis, 20 μg of total RNA were denatured with formaldehyde and electrophoresed in 1% agarose gel in the presence of formaldehyde. The RNA was transferred to nylon membranes (Boehringer Mannheim, East Sussex, UK) by capillary blotting with 20 × standard sodium citrate (SSC) for 18 hours and fixed by UV irradiation. For hybridization, a fragment of the LDL receptor cDNA probe, and as control, glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA were used. The LDL receptor cDNA probe (2.8 kb) was prepared by digesting the plasmid pLDLR3 (American Type Culture Collection, Rockville, MD, USA) with the restriction endonucleases *Hin*dIII and SmaI. The GAPDH cDNA probe (0.8 Kb) was prepared by digesting plasmid pHcGAP (American Type Culture Collection, Rockville, MD, USA) with the restriction endonucleases Pst I and Xba I. The cDNA probes were labeled with $^{32}$P-dCTP (3000 Ci/mmol; Amersham, Buckinghamshire, UK) by nick translation. The labeled probes were separated from unincorporated nucleotides by Sephadex G-50 columns (Pharmacia, Herts, UK). Prehybridization with cDNA probes were performed in prehybridization buffer [5 × SSPE, 5 × Denhardt's, 100 μg/mL hsDNA (herring sperm DNA), 50% deionized formamide] for two hours at 42°C. The blots were hybridized in hybridization buffer (the same as the prehybridization buffer except that 1 × Denhardt's was used) overnight at 42°C. The filters were washed twice for 5 minutes in 2 × SSC with 0.1% sodium dodecyl sulfide (SDS) at room temperature, followed by two 15-minute high stringency washes in 0.1 × SSC containing 0.1% SDS at 68°C.

### RT-PCR

Total RNA (500 ng) was used as a template for the reverse transcription-polymerase chain reaction (RT-PCR). All reagents were obtained from Perkin-Elmer (PE Applied Biosystems Ltd., Warrington, Cheshire, UK). The RT reaction was set up in a 20 μL mixture containing 50 mmol/L KCl, 10 mmol/L Tris/HCl, 5 mmol/L $MgCl_2$, 1 mmol/L of each dNTPs, 2.5 μmol/L random hexamers, 20 U RNAsin, and 50 U of Moloney-murine leukemia virus (M-MLV) reverse transcriptase. Incubations were performed in a DNA Thermal Cycler (Perkin-Elmer 9600) for 10 minutes at room temperature, followed by 30 minutes at 42°C and 5 minutes at 99°C. After cDNA synthesis by RT, the incubation mixture was split into two 10-μL aliquots for separate amplification of the SCAP cDNA and the GAPDH cDNA using specific primers. SCAP 5′ primer (nucleotide position 2574 to 2595); GCCCTCCGCCGCCTTCCCTCTT, 3′ primer (nucleotide position 2933-2953); GCTGCTCCGCCCC ACCACGAT [4]. GAPDH: 5′ primer (nucleotide position 73 to 92) TCATAGACAAGATGGTGAAG, 3′ primer (nucleotide position 303 to 327) TGACGGGAT CTCGCTCCTGGAAGAT. For PCR, the final concentrations of the PCR reaction mixture were 50 mmol/L KCl, 10 mmol/L Tris/HCl, 2 mmol/L $MgCl_2$, 200 μmol/L dNTPs, 0.125 μmol/L of primers, 1.25 U *Taq* DNA polymerase. After incubation for 145 seconds at 95°C, 26 cycles were performed for 30 seconds at 95°C, 30 seconds at 65°C, and 60 seconds at 72°C. Twenty microliters of each PCR reaction were subjected to electrophoresis in a 2% agarose gel.

### Southern blot analysis and quantitative evaluation

Nucleic acids were transferred to a nylon membrane (Boehringer Mannheim, Lewes, East Sussex, UK) using the Southern analysis method. These membranes were probed with [γ-$^{32}$P]ATP (3,000 Ci/mmol, Amersham, Little Chalfont, Buckinghamshire, UK)-labeled oligonucleotides. The SCAP probe (nucleotide position 2654 to 2676): GGGCTGAGTGGGCTGTGAGGA [4] and GAPDH probe (nucleotide position 170 to 191): AATGAAGGG GTCGTTGATGGCA were labeled using 5′-end labeling system (Promega, Southampton, Hampshire, UK). Membranes were irradiated by UV Stratalinker for 3 minutes, prehybridized in 50% formamide, 5 × Denhardt's solution, 5 × SSPE, 100 μg/mL hsDNA (herring sperm DNA), 50 mmol/L sodium phosphate (pH 6.8) at 42°C for 4 hours, and hybridized overnight at 42°C with at least 1 × $10^6$ cpm/mL of the labeled probe in the same solution as for prehybridization but without Denhardt's reagents. The membranes were washed twice for 15 minutes at room temperature with 5 × SSC, and then washed for 10 minutes at 42°C with 5 × SSC. The blots were exposed to x-ray films (Eastman Kodak, Rochester, NY, USA) for four hours. Relative radiation density (the ratio of SCAP to GAPDH) was calculated for each sample to adjust for the differences in RNA mass between templates and was used for the quantitative comparisons.

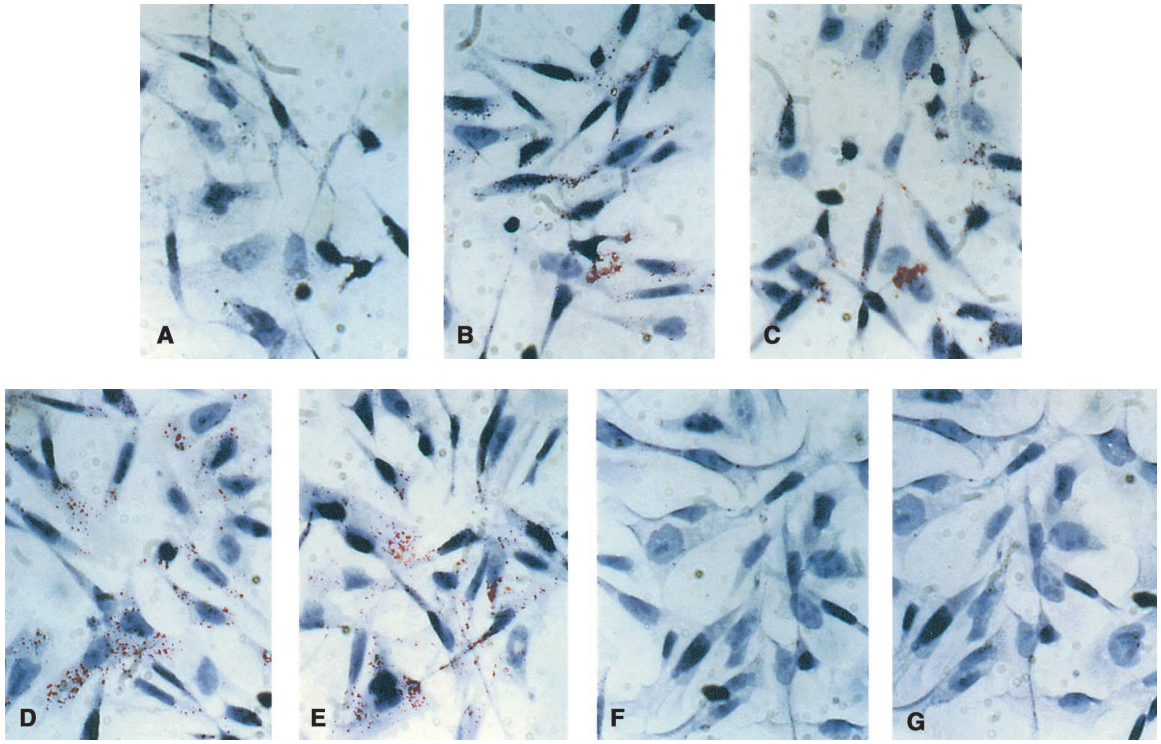


**Fig. 1. Visualization of low-density lipoprotein (LDL) uptake and lipid droplets in a human mesangial cell line (HMCL) after tumor necrosis factor-α (TNF-α) or interleukin-1β (IL-1β) treatment.** HMCLs were incubated for 24 hours in serum-free medium with 250 μg/mL of native LDL in the absence (*A*) or presence of 50 ng/mL of TNF-α (*B*), 5 ng/mL of IL-1β (*C*), TNF-α plus Poly I (*D*), IL-1β plus Poly I (*E*), TNF-α plus heparin (*F*), and IL-1β plus heparin (*G*). The cells were examined for lipid inclusions by Oil Red O staining. The results are typical of those observed in four separate experiments (×400).

### Preparation of nuclear fraction

To prepare nuclear extracts, cells from duplicate 75 $cm^2$ culture flasks were pooled and allowed to swell at 4°C for 30 minutes in 1.5 mL of buffer A [10 mmol/L HEPES, pH 7.9, 10 mmol/L KCl, 1.5mmol/L $MgCl_2$, 0.5 mmol/L dithiothreitol (DTT), 0.4% NP-40, 0.5 μmol/L phenylmethylsulfonyl fluoride (PMSF), and 1 μg/mL of antipain, laupeptin, bestatin, and chymostatin], and then passed through a 23 gauge needle 20 times before centrifugation at 1000 × *g* at 4°C for 7 minutes. The 1000 × *g* pellet was resuspended in 60 μL of buffer B (20 mmol/L HEPES pH 7.9, 0.42 mol/L NaCl, 1.5 mmol/L $MgCl_2$ 25% glycerol, 0.2 mmol/L EDTA, 0.5 mmol/L DTT, 0.5 μmol/L PMSF, 2 mmol/L benzamidine and 1 μg/mL of antipain, laupeptin, bestatin, and chymostatin). The suspension was passed through a 23 gauge needle 20 times, incubated for 30 minutes at 4°C, then centrifuged at 13,000 rpm in a microcentrifuge for 15 minutes at 4°C. The supernatant from this spin was used as the nuclear extract.

### Western blot analysis for SREBP-1

Identical amounts of total protein from nuclear extracts were denatured, then subjected to electrophoresis on a 5% stacking and 8% separating SDS polyacrylamide gel in a Bio-Rad mini Protein II apparatus. Electrophoretic transfer to nitrocellulose was accomplished at 100 V, 350 mA for one hour in 25 mmol/L Tris, pH 8.3, 192 mmol/L glycine, 0.1% SDS and 20% methanol. The membrane was then blocked with 3% blocker (Bio-Rad Laboratories, Herts, UK) for one hour at room temperature followed by two five-minute washes in PBST (phosphate-buffered saline/1% Tween-20). The membrane was incubated with rabbit anti-human SREBP-1 polyclonal antibody (1 μg/mL; Santa Cruz Biotechnology) for one hour in antibody dilution buffer (1% BSA in PBST) followed by two five-minute washes in PBST. A goat anti-rabbit HRP-labeled antibody (Bio-Rad Laboratories) was diluted in antibody dilution buffer, then added to the membrane for one hour followed by two five-minute washes in PBST. Finally, amplification and colorimetric detection procedures were performed using a Bio-Rad Opti-4CN detection Kit (Bio-Rad Laboratories).

### Data analysis

In all experiments, groups of data were evaluated for significance by one-way analysis of variance (ANOVA) using Minitab software. Data were considered significant if the $P$ value $\leq 0.05$.

## RESULTS

Experiments were performed to determine whether LDL is able to cause foam cell formation in the presence

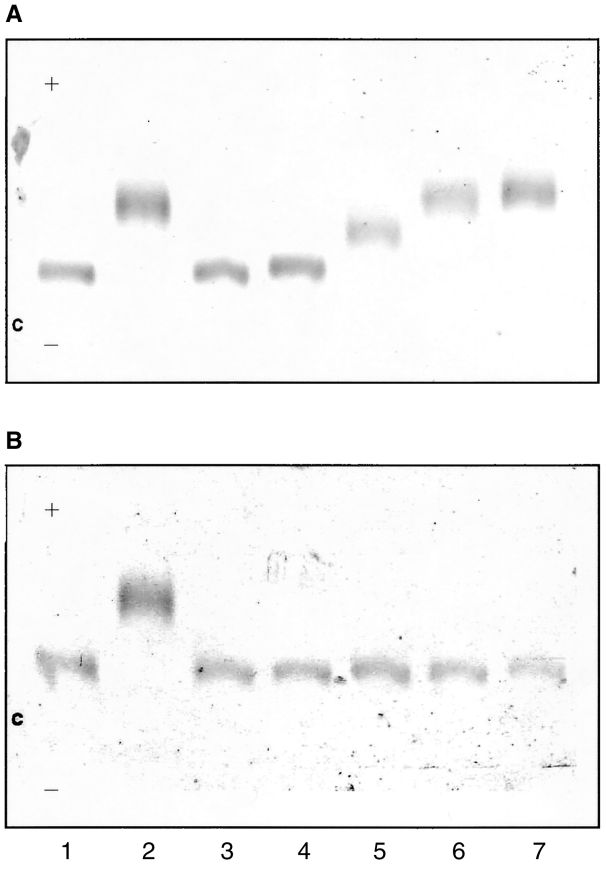


**Fig. 2. Electrophoretic mobility of LDL from the culture medium.** HMCL were incubated for 24 hours in serum-free RPMI-1640 medium with 250 μg/mL of native LDL in the absence (*A*) or presence of 100 μmol/L of EDTA and 20 μmol/L of BHT (*B*) for the indicated time periods. The supernatants were collected and subjected to electrophoresis in Beckman Paragon LIPO Gel. Lane 1, fresh native LDL (negative control); lane 2, Ox-LDL (positive control); lane 3, 0 hour incubation with HMCL; lane 4, 24 hours; lane 5, 48 hours; lane 6, 72 hours; lane 7, 96 hours.

of cytokines. Staining of HMCL with Oil Red O before (Fig. 1A) and after 50 ng/mL of TNF-α (Fig. 1B) and 5 ng/mL of IL-1β (Fig. 1C) stimulation in the presence of a high concentration of native LDL (250 μg/mL) and antioxidants (100 μmol/L of EDTA and 20 μmol/L BHT) showed that both cytokines increased the number of intracellular Oil Red O-stained lipid droplets. This process was not inhibited by polyinosinic acid (Poly I), which blocks scavenger receptors (Fig. 1 D, E) [14], but was prevented by heparin, which removes LDL bound to the cell surface (Fig. 1 F, G) [15]. The electrophoretic mobility of LDL from the culture medium was the same as that of fresh LDL excluding the participation of oxidized LDL and scavenger receptor (Fig. 2). This also suggested that anti-oxidants were unable to prevent foam cell formation in the presence of inflammatory cytokines.

The influence of cytokines on the regulation of LDL receptor expression was examined by flow cytometry. TNF-α (50 ng/mL) and IL-1β (5 ng/mL) increased LDL uptake by HMCL in the presence of a high concentration of native LDL and anti-oxidants (100 μmol/L of EDTA and 20 μmol/L BHT; Fig. 3). These observations suggest that TNF, and to a greater extent IL-1β, overrode the LDL receptor protein suppression induced by a high concentration of native LDL.

We have previously examined LDL receptor promoter activity using a reporter gene pGL3LDLR6500 in HMCL [12]. A high concentration of native LDL (250 μg/mL) markedly decreased promoter activity. However, TNF-α (50 ng/mL) and IL-1β (5 ng/mL) increased LDL receptor promoter activity even in the presence of a high concentration of native LDL [12]. The present study examined LDL receptor mRNA expression in HMCL cultured in a high concentration of native LDL (250 μg/mL), LDL with TNF-α (50 ng/mL) or IL-1β (5 ng/mL). Both cytokines significantly increased LDL receptor mRNA expression, even in the presence of a high concentration of native LDL (Fig. 4). This result suggests that inflammatory cytokines increase LDL receptor expression at a transcriptional level, even in the presence of high concentrations of cholesterol.

Sterol regulatory element binding proteins SREBPs are central molecules in the control of LDL receptor regulation. SREBP-1 translocation was investigated from the cytoplasm to the cell nucleus in the presence of LDL and inflammatory cytokines. Nuclear SREBP-1 was detected by the SREBP-1–specific polyclonal antibody using Western blotting. A high concentration of LDL (200 μg/mL) decreased SREBP-1 level in the nucleus in a time-dependent manner. However, both inflammatory cytokines TNF-α (50 ng/mL) and IL-1β (5 ng/mL) increased the level of nuclear SREBP-1 in the presence of a high concentration of LDL (Fig. 5).

Finally, the mRNA expression of SCAP in HMCL was examined under the influence of high concentrations of cholesterol. Cholesterol depletion up-regulated SCAP expression, whereas high concentrations of native LDL down-regulated SCAP mRNA expression in a time-dependent manner (Fig. 6). This suggests that intracellular concentrations of cholesterol affect SCAP expression at the transcriptional level and that SCAP is a cholesterol sensor that regulates LDL receptor expression in HMCL. We also showed that LDL down-regulated SCAP mRNA expression in a dose-dependent manner in both HMCL and primary cultures of HMC (Fig. 7.). TNF-α and IL-1β increased SCAP mRNA expression in HMCL in a dose responsive manner, even in the presence of high concentrations of cholesterol (Fig. 8). These results indicate that overexpression of SCAP may be an important factor in overriding the suppression of native LDL receptor induced by high concentrations of cholesterol through the cleavage of SREBP-1.

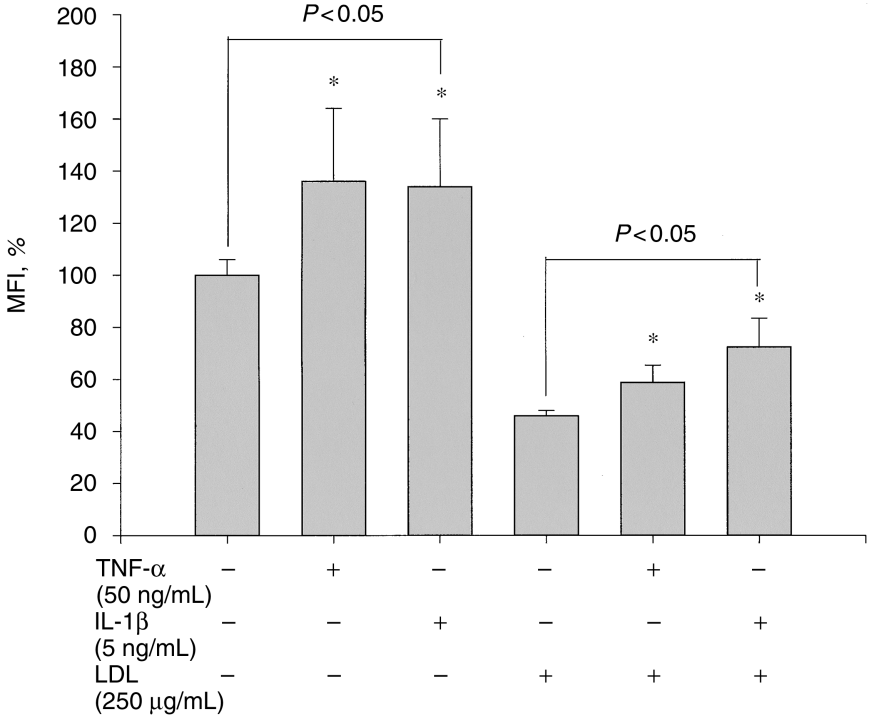


**Fig. 3. Analysis of the mean fluorescence intensity (MFI) in the TNF-α- or IL-1β-treated HMCL.** HMCL were incubated in serum-free medium alone or with 50 ng/mL of TNF-α or 5 ng/mL of IL-1β in the absence or presence of a high concentration of native LDL (250 μg/mL) for 24 hours. The medium was then replaced by fresh serum-free medium containing 10 μg/mL DiI-labeled LDL for 5 hours at 37°C. The cells were analyzed by FACS. MFI was calculated by subtracting the autofluorescence intensity from the observed MFI of labeled cells (B). Results represent means ± SD of duplicate wells from four experiments. *$P < 0.05$ vs. control.

## DISCUSSION

In vivo, foam cell formation was firstly described in macrophages. During the development of the lesions of atherosclerosis, monocytes became adherent to the surface of the endothelium, migrated subendothelially, and accumulated lipid [16]. In the kidney, the formation of glomerular foam cells is encountered in renal glomeruli in cases of nephrotic syndrome and in renal allografts [17]. It is generally accepted that lipid-laden cells identified by Oil Red O staining in vitro are the equivalent of foam cells.

Our previous studies showed that the inflammatory cytokines TNF-α, TGF-β, PDGF, and IL-1β increased LDL receptor gene expression in HMC by a sterol- and proliferation-independent gene transcription pathway in a human mesangial cell line [12]. Using the Oil Red O staining technique, the present studies extend these observations to show that feedback regulation prevents native LDL from causing foam cell formation when HMCL are incubated with native LDL. However, TNF-α and IL-1β override the suppression of LDL receptor induced by a high concentration of LDL. The process by which TNF-α and IL-1β increased the number of intracellular Oil Red O-stained lipid droplets in HMCL could not be inhibited by Poly I, which blocks scavenger receptors [14], but was blocked by heparin, which removes LDL bound to the cell surface [15], implying LDL receptor pathway involvement and effectively excluding the participation of scavenger receptor. Although both polyinosinic acid and heparin are not specific blockers of scavenger and LDL receptor respectively, they have been widely used in the study of oxidized or native LDL binding and internalization. Additionally, all experimental incubation media contained the antioxidants EDTA and BHT, both of which powerfully prevent oxidation of LDL by HMCL. The electrophoretic mobility of LDL from the culture medium was the same as that of fresh LDL, indicating that no oxidation had taken place during the experiments. Therefore, there was no ligand for scavenger receptors in the culture medium. Taken together, these results imply that foam cell formation occurred through the dysregulation of the LDL receptor.

In the classic pathway of cholesterol homeostasis, cholesterol is the major regulatory element. Intracellular levels of cholesterol control the uptake and synthesis of cholesterol through feedback regulation [1]. The evidence presented in our study, together with our previous studies of promoter activity [12], show that normal feedback regulation is preserved in HMCL in culture, demonstrating the functional integrity of the LDL receptor. The present study provides experimental evidence to suggest that inflammatory cytokines may independently modulate LDL receptor function. Therefore, it appears that the normally tight sterol-dependent feedback LDL receptor regulation in HMC is ineffective under the influence of TNF-α and IL-1β. Thus native LDL can be taken up in excess via LDL receptors to convert HMCL into foam cells.

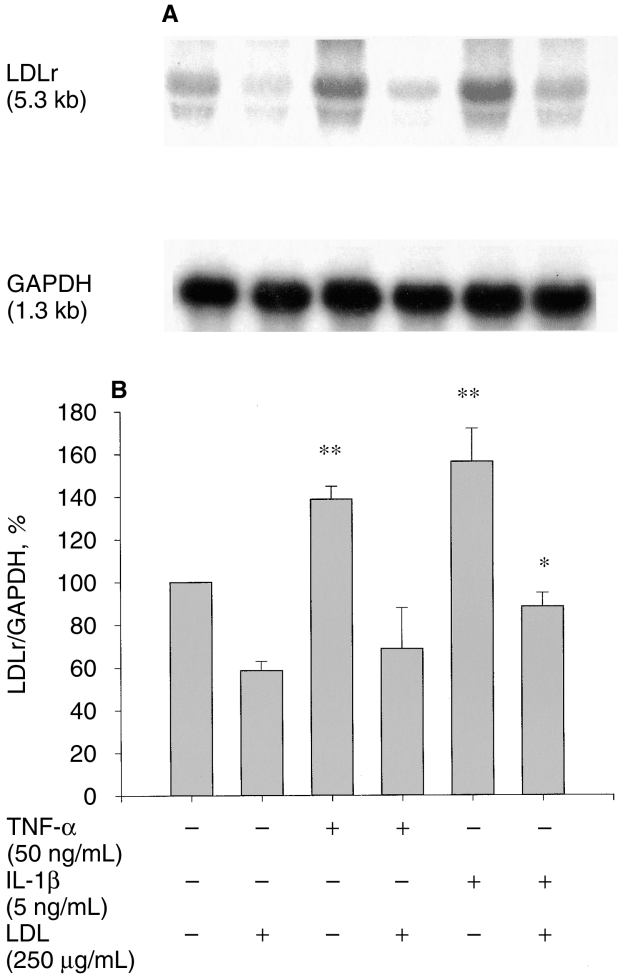


**Fig. 4. Inflammatory cytokines overrode the LDL receptor mRNA suppression induced by a high concentration of native LDL.** HMCL were incubated in serum-free medium alone (control) or with 50 ng/mL of TNF-α or 5 ng/mL of IL-1β in the absence or presence of 250 μg/mL of native LDL for 24 hours. (*A*) LDL receptor mRNA expression was examined using Northern blotting as described in the **Methods** section. (*B*) Means ± SD of the densitometric scans of the LDL receptor mRNA band from three experiments, normalized by comparison with GAPDH mRNA, and expressed as a percentage of control. *$P < 0.05$ vs. control + LDL group; **$P < 0.001$ vs. control.

We also investigated the molecular mechanisms by which inflammatory cytokines overrode the normal cholesterol-mediated suppression of the LDL receptor by examining the expression of human SCAP. The high expression of SCAP mRNA in sterol-depleted HMCL was suppressed by high concentrations of LDL, suggesting that SCAP contributed to LDL receptor regulation by acting as a cholesterol sensor. Both inflammatory cytokines TNF-α and IL-1β increased SCAP mRNA expression in the presence of high concentrations of LDL. This result suggests that inflammatory cytokines are able to regulate SCAP expression at a transcriptional level. Up-regulation of SCAP stimulates cleavage of SREBPs, which enter the nucleus and promote LDL receptor transcription.

Our observations demonstrate a potential new pathway for foam cell formation and suggest an important mechanism through which inflammation might potentiate lipid-mediated glomerular injury. This provides a possible explanation for the observation that abnormal glomerular deposition of LDL cholesterol is found in various inflammatory renal diseases [18]. Nevertheless, foam cell formation is not always a recognized feature of glomerulonephritis, possibly because the pathogenesis of glomerulonephritis and glomerulosclerosis is complex. Foam cell formation may be an early event that is easily missed, especially since routine de-fatting of renal biopsy samples destroys foam cell appearance. Additionally, foam cells may be difficult to find in the final stages of glomerulosclerosis because of matrix expansion and fibrosis. Our data also emphasize that foam cell formation may be influenced by the underlying inflammatory milieu within the kidney, which varies in different disease states and at different times. This possibility clearly merits further investigation. Finally, our findings may partly explain the ineffectiveness of cholesterol-lowering strategies in arresting the progression of renal disease.

Recent studies have linked systemic infection and inflammation with atherosclerosis [9, 19, 20]. Furthermore, local inflammation occurs at the site of plaque formation. Patients with elevated levels of acute-phase reactants have a less favorable clinical course than those with normal levels of acute-phase reactants. Acute-phase reactants are elevated in many chronic diseases; for example, Janssen et al reported that C-reactive protein (CRP) and complement C3a levels are significantly raised in patients with IgA nephropathy [21].

Our current understanding of the association between inflammation and atherosclerosis is primarily based on "the response to injury hypothesis," which suggests that inflammation causes endothelial injury and mediates hemodynamic damage [22]. However, the observations we have reported suggest that inflammatory cytokines can modify cholesterol homeostasis through the dysregulation of the LDL receptor. Such dysregulation would permit uncontrolled accumulation of LDL within cells. In the kidney, this process may specifically contribute to progressive renal disease and chronic renal transplant dysfunction. In arteries, the same process may result in atherosclerosis. The implications of these findings are that inflammatory cytokines are risk factors for atherogenesis, and that no cholesterol concentration is "safe" in the presence of acute or chronic inflammation. Therefore, anti-inflammatory drugs may be useful therapeutic agents in addition to anti-oxidants and cholesterol-lowering drugs.

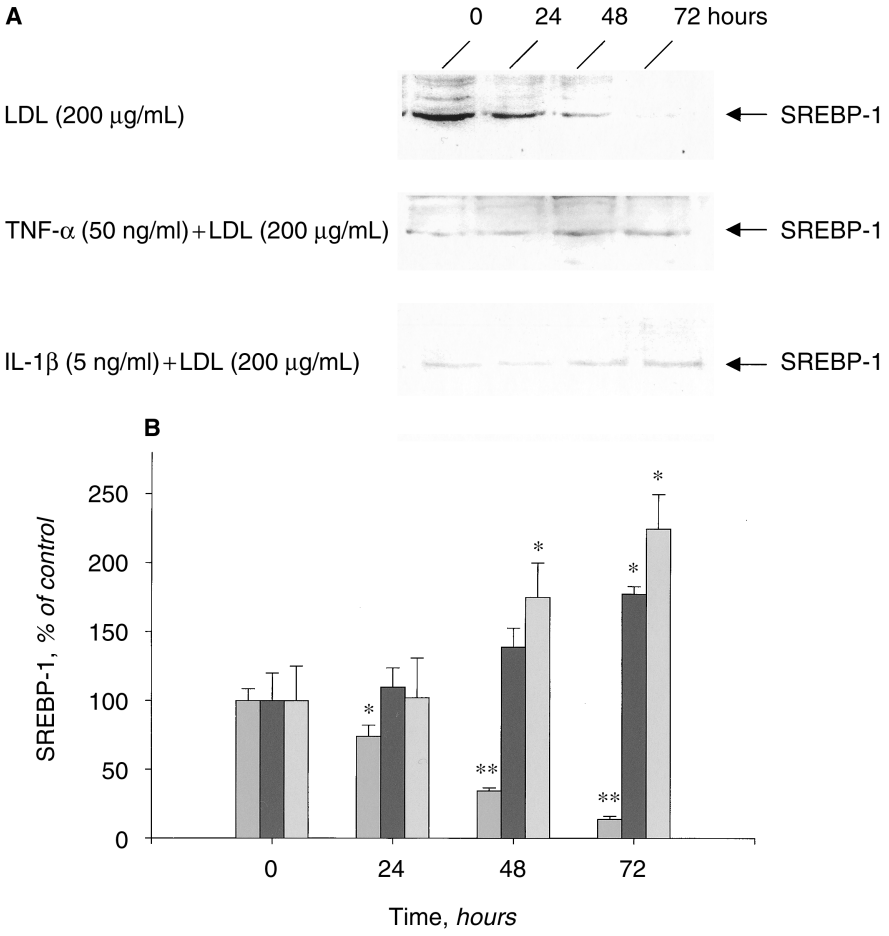


**Fig. 5. Inflammatory cytokines increased sterol-responsive element binding protein (SREBP) cleavage in the human mesangial cell line (HMCL).** HMCL were incubated in serum-free medium or serum-free medium containing 200 μg/mL of LDL in the presence of 50 ng/mL of TNF-α or 5 ng/mL of IL-1β for 0 (control), 24, 48, and 72 hours. The nuclear extracts were prepared and subjected to SDS-PAGE, followed by immunoblot analysis. (*A*) The SREBP-1 protein level was examined using Western blotting as described in the **Methods** section. (*B*) Means ± SD of the densitometric scans of the SREBP-1 protein bands from three experiments and expressed as a percentage of control. Symbols are: (□) LDL; (■) TNF-α + LDL; (▩) IL-1β + LDL. $*P <$ 0.05 vs control, $**P < 0.001$vs control.

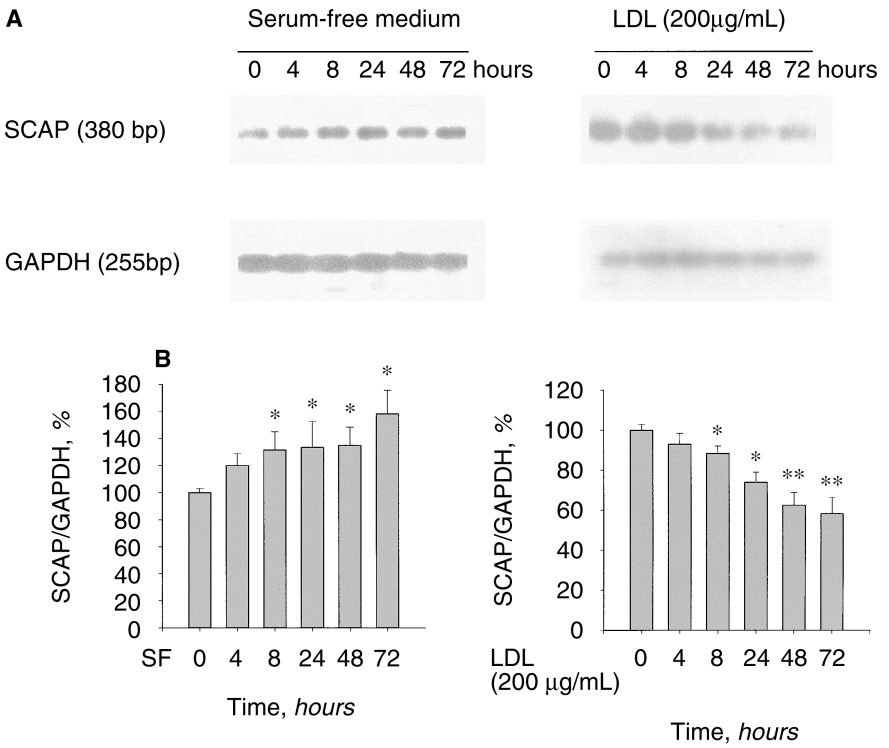


**Fig. 6. Intracellular concentrations of cholesterol regulated SREBP cleavage activation protein (SCAP) mRNA expression in a time-dependent manner in HMCL.** HMCL were incubated in serum-free medium alone or serum-free medium with 200 μg/mL of LDL for 0 (control), 4, 8, 24, 48, and 72 hours. (*A*) SCAP mRNA expression was examined using RT-PCR followed by Southern blotting as described in the **Methods** section. (*B*) Means ± SD of the densitometric scans of the SCAP mRNA band from three experiments, normalized by comparison with GAPDH mRNA, and expressed as a percentage of control. $*P < 0.05$ vs. control, $**P < 0.001$ vs. control.

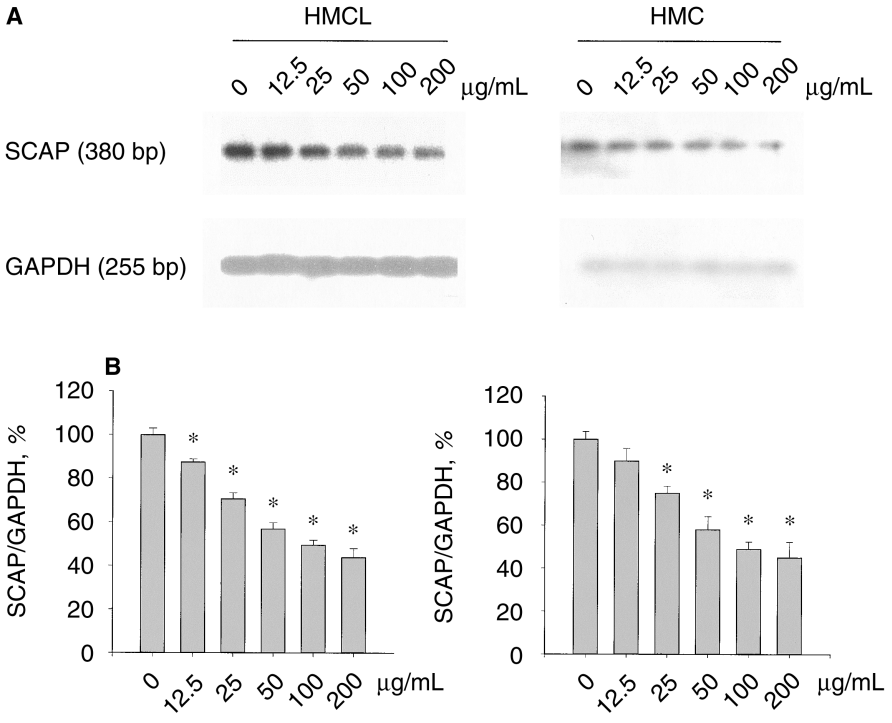


**Fig. 7. Intracellular concentrations of cholesterol regulated SCAP mRNA expression in a dose-responsive manner in both HMCL and primary culture of HMC.** Both HMCL and HMC were cultured serum-free medium (control) or serum-medium containing various concentrations of native LDL (0, 12.5, 25, 50, 100, and 200 μg/mL) for 24 hours. (*A*) SCAP mRNA expression was examined using RT-PCR followed by Southern blotting as described in the **Methods** section. (*B*): The means ± SD of the densitometric scans of the SCAP mRNA band from three experiments, normalized by comparison with GAPDH mRNA, and expressed as a percentage of control. $*P < 0.001$ vs. control.

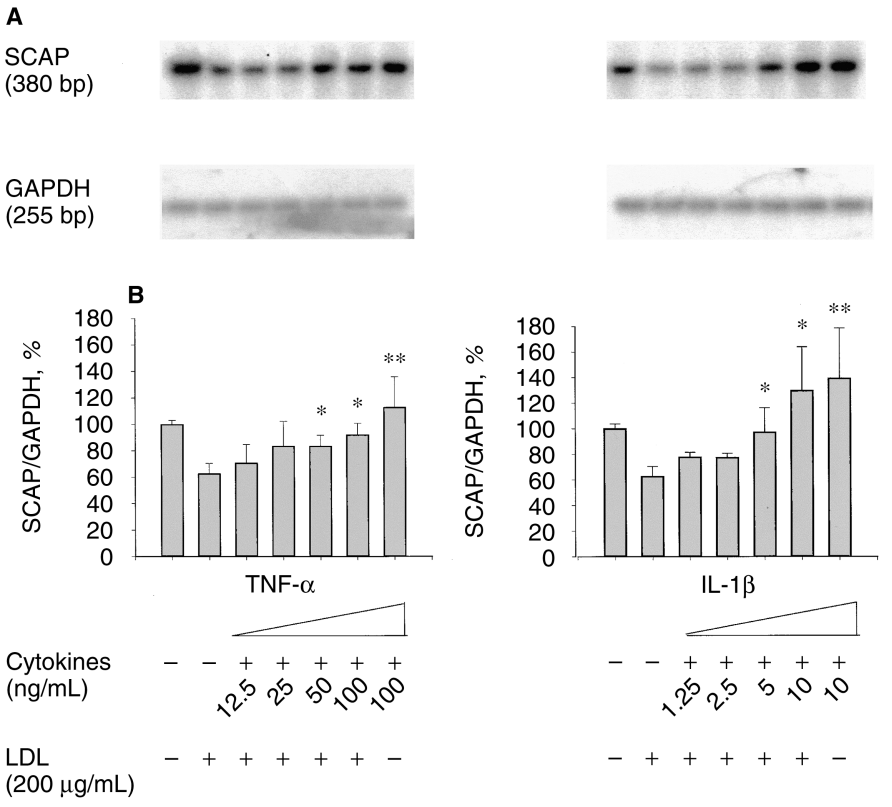


**Fig. 8. Inflammatory cytokines increased SCAP mRNA expression in HMCL.** HMCL were incubated in serum-free medium alone (control) or with 200 μg/mL of native LDL in the absence or presence of various concentrations of TNF-α or IL-1β for 24 hours. (*A*) SCAP mRNA expression was examined using RT-PCR followed by Southern blotting as described in methods. (*B*) Means ± SD of the densitometric scans of the SCAP mRNA band from three experiments, normalized by comparison with GAPDH mRNA, and expressed as a percentage of control. $*P < 0.05$ vs. control + LDL group, $**P < 0.05$ vs. control.

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*Reprint requests to Dr. Xiong Z. Ruan, Centre for Nephrology, Royal Free and University College Medical School, University College London, Royal Free Campus, Rowland Hill Street, London NW3 2PF, England, United Kingdom.*
*E-mail: xzruan@rfc.ucl.ac.uk*

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